

THE MEASUREMENT OF GASTRIC ACID

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HUMAN gastric juice contains water, hydrochloric acid, pepsin, intrinsic factor, mucus and electrolytes. Of these, hydrochloric acid has been studied most extensively by the clinical investigator—partly because of the likely association of its erosive powers with the development of peptic ulceration, and partly because of the apparent simplicity of its estimation.

Test Meals

Since the end of the last century the standard method of assessing gastric acid secretion has been by the test meal, which was developed largely by von Leube (1876), Ewald and Boas (1885) and Rehfuß (1927). Its history and development have been reviewed by Hollander and Penner (1939) and Rovelstad (1963). The vast amounts of data collected in these original studies contributed only little to our knowledge of the secretions of the stomach in health and in disease. With the recent development of precise quantitative methods of assessing gastric function, such investigations are now largely of historical interest.

The 'fractional test meal', still performed today, differs little from that described by Rehfuß in 1914. Following a fast, a gastric tube is passed and the stomach emptied. A 'test meal' of carbohydrate—bread, cereal, gruel or biscuits, is ingested and 10 ml. of gastric juice aspirated each 15 minutes for 2-3 hours. The acidity in each sample is estimated by titration with 0.1 N NaOH using two indicators—Topfer's dimethylaminoazobenzene for 'free', and phenolphthalein for 'total' acid. The acidity curve is drawn on a chart which shows a 'normal range' based on the curves of 80 of the 100 normal students studied by Bennett and Ryle in 1921. It is of interest that the other 20 students showed curves from extreme hypo- to hypersecretion.

The fractional test meal has little to commend it as a test of gastric function. It is

neither precise, quantitative nor reproducible. The coefficient of variation of the concentration of free acid in a series of 20 consecutive meals performed on a healthy adult ranged from 25.8 to 199.6%—quite outside acceptable limits (Bell and McAdam, 1924). It is therefore not surprising that Enticknap and Merivale (1954), in an analysis of 1000 gruel meals, concluded that they were of little diagnostic value. In a comparison with the augmented histamine test in 114 patients, Marks and Shay (1960) found that, although the peak acidity in a fractional test meal was statistically related to the acid output in the augmented histamine test, the results of a test meal could not be used to predict the true acid output in an individual patient.

As the 'test meal' was a poor stimulus of gastric secretion, differing little from plain water (Bergeim, Rehfuß and Hawk, 1914), the incidence of achlorhydria was fairly high. More potent stimuli were used—alcohol (Kast, 1906; Ehrmann, 1912) caffeine (Katsch and Kalk, 1924) and histamine, either in single (Bockus and Bank, 1927; Vanzant, Alvarez, Eustermen, Runn and Berkson, 1932), double (Rivers, Osterby and Vanzant, 1936), or triple (Hitchcock, Sullivan and Wangenstein, 1955) doses. However, even with a standard dose of histamine (usually 0.01 mg. histamine acid phosphate per kg. body weight) the response had a wide variation in normal subjects and proved of little routine diagnostic value (Polland and Bloomfield, 1931; Polland, 1933).

Criteria of Tests of Acid Secretion

Collections

The measurement of a secretory response requires an estimate of both the volume of juice secreted and its acidity. Therefore, the gastric tube must be placed so that *all* the juice secreted is withdrawn for analysis. 'Blind' placement of gastric tubes is unsatisfactory (Callender, Retief and Witts, 1960) and radio-

logical screening is now generally considered to be an essential part of any test.

Efficient aspiration is necessary for complete collection of the juice secreted. Using a radio-opaque marker as an index of complete aspiration, Johnston and McGraw (1958) found suction by syringe more efficient than by a pump, but continuous aspiration by low-pressure suction pump is now more generally used. This requires constant care to ensure that the tube remains unblocked—either by the repeated injection of air down the tube, or by the use of an 'air vent' in the pump or in the tube.

Titration

The concentration of acid in the specimens of gastric juice must be accurately estimated. Gastric juice, during adequate stimulation of secretion, has a pH of approximately 1.0. In this range of acidity the measurement of pH inaccurately reflects change in titratable acidity. For example, at pH 1 a change of 0.1 pH unit is equivalent to 26 mEq. HCl/l, while at a pH of 3 a change of 0.1 pH unit represents a change of only 0.5 mEq. HCl/l (James, 1957). The best means of determining acid concentration is by titration to 'neutrality'.

The concept of free and combined acid arose from a study of the responses to test meals (Prout, 1824; Michaelis, 1926) when a proportion of the acid is buffered by the ingested meal itself. The titration curve of the aspirated gastric juice resembled that of pure hydrochloric acid only in the range of pH 1-3. It was assumed that titration to a pH of 2.8-3.0 could be attributed to 'free' hydrochloric acid. This was determined using Topfer's reagent (dimethylaminoazobenzene) which changes colour at this pH. Further titration to neutrality followed a different curve and this was considered to be due to 'combined' acid, and estimated with phenolphthalein (colour change pH 8.0-10.0).

The titration curve of uncontaminated gastric juice does not, in fact, differ from that of hydrochloric acid and these terms should be abandoned in favour of total titratable acidity (Bock, 1962).

Accurate titration of gastric juice to neutrality is best performed electrometrically. With the universal availability of the glass-electrode pH meter this is simpler and more accurate than by the use of indicators (Berk, Thomas and Rehfuss, 1942). Although at 37°C the isoelectric point of HCl is pH 6.8, in practice, titration to a pH of 7.0 is satisfactory.

Expression of results

Acidity is frequently expressed in terms of normality. As a normal solution of HCl contains 1000 mEq/l., millinormality (mN) can also be expressed as mEq./l. The 'clinical unit' still occasionally referred to is the ml. 0.1 N NaOH required to titrate 100 ml. of gastric juice. This is equivalent to the *millinormality* or the number of milliequivalents per litre.

The output of acid secreted by the stomach in mEq./unit time (usually one hour) is the product of the volume (in litres) and the concentration (in mEq./l).

Secretory rate

Apart from the accuracy in collection and estimation of acidity, the output of acid determined is dependent upon the rate of secretion and therefore upon the degree of stimulation of the parietal cells, which may vary from a basal state to one of maximal secretory activity.

Basal Secretion

In man it is difficult to achieve truly basal conditions. Psychogenic factors are known to stimulate, through the vagus, acid gastric secretion. Basal juice may be collected overnight (Levin, Kirsner, Palmer and Butler, 1948a; Sandweiss, Friedman, Sugarman and Podolsky, 1964a and b; Johnson, 1962) or as a timed collection after an overnight fast (Levin, Kirsner and Palmer, 1950). In general patients with duodenal ulcer have a higher unstimulated acid output than normals, but there is a wide overlap. Considerable day to day variation also occurs in individual patients (Levin, Kirsner, Palmer and Butler, 1948b).

Very high levels of basal secretion (a basal 1-hour secretion of 15 mEq. HCl or more, or a 12-hour night secretion of 2 litres and 120 mEq. (Sircus, 1962)) should always raise the suspicion of a Zollinger-Ellison tumour.

Stimulated Secretion

The augmented histamine test

Histamine strongly stimulates acid gastric secretion in experimental animals (Popielski, 1920) and in man (Carnot, Koskowski and Liebert, 1922). Increasing doses produce an increasing response and Kay (1953) has shown that a single dose of 0.04 mg. histamine acid phosphate per kg. body weight will induce 'maximal' secretion. The side effects of a dose

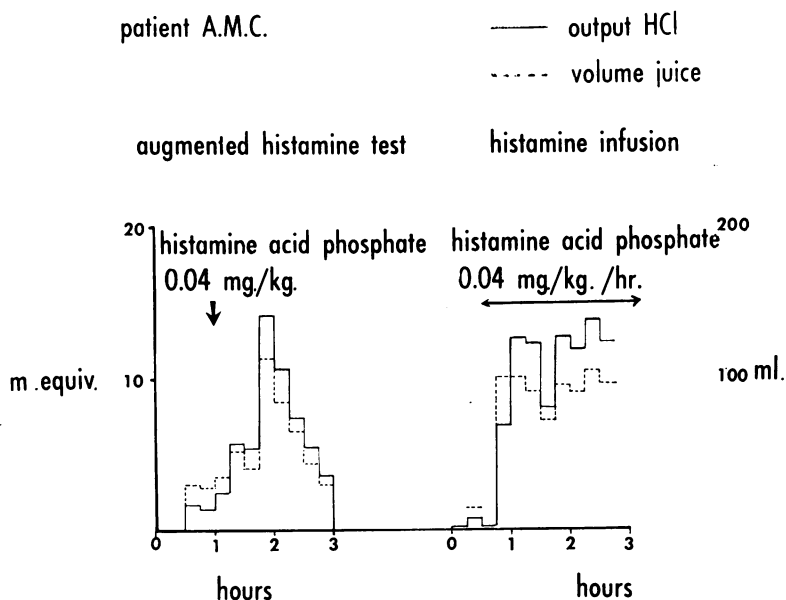


FIG. 1.—Augmented histamine test—acid output per 15 minutes, with an infusion test in the same patient.

of this magnitude are prevented by the prior administration of an anti-histamine which is without effect on the action of histamine on the parietal cell (Conrad, Kowalewski and Geertruyden, 1949; Kay, 1953).

Following intubation of the patient the basal secretion is collected and 100 mg. mepyramine maleate given intramuscularly after 30 minutes. Thirty minutes later (i.e. after one hour of basal collection) histamine acid phosphate, 0.04 mg./kg. body weight, is injected subcutaneously and collections continued for four 15-minute periods. The volume and acidity of each specimen is estimated and the output of HCl calculated in milliequivalents.

As this large dose of histamine induces total parietal cell activity, the response is related to the parietal cell mass (Card and Marks, 1960; Marks, Komarov and Shay, 1960). It is therefore reproducible and similar figures for normal individuals and patients with duodenal ulcer have been reported from different centres (Marks, 1961). Nevertheless, it is a transient response which reaches a peak normally during the second or third 15-minute period after the injection of histamine and then declines to basal levels (Fig. 1). Thus, though the dose of histamine used induces maximum secretion, this is only achieved for a limited period of time within the total response.

Various workers have used different com-

binations of the 15-minute collection to express the results. These are usually quoted as the output of HCl in mEq./hour. The average output of acid in normal subjects is 22-23 mEq./hour and that for patients with duodenal ulcer 37-40 mEq./hour (Marks, 1961).

Histamine infusion test

Many of the shortcomings of the augmented histamine response can be overcome if a steady state of secretory activity is induced by a continuous infusion of histamine. In dogs, this stimulus has been widely used to study the function of gastric pouches but few reports have been made on its use in man (Adam, Card, Riddell, Roberts, Strong and Woolf, 1954; Hirschowitz, London and Wiggins, 1957). By performing dose-response curves using this stimulus we have shown that a maximal plateau can be obtained with a dose of histamine acid phosphate of 0.04 mg./kg. body weight/hour (Lawrie, Smith and Forrest, 1964).

With the recent availability of pre-sterilised plastic equipment and slow-injection infusion pumps, a histamine infusion can now be used for the routine assessment of the secretion of gastric acid in man. The administration of this relatively small dose of histamine by continuous intravenous infusion, though inducing maximal response from the stomach, elicits fewer side-effects than a single large dose. A small dose of anti-histamine (25-50 mg.) adequately miti-

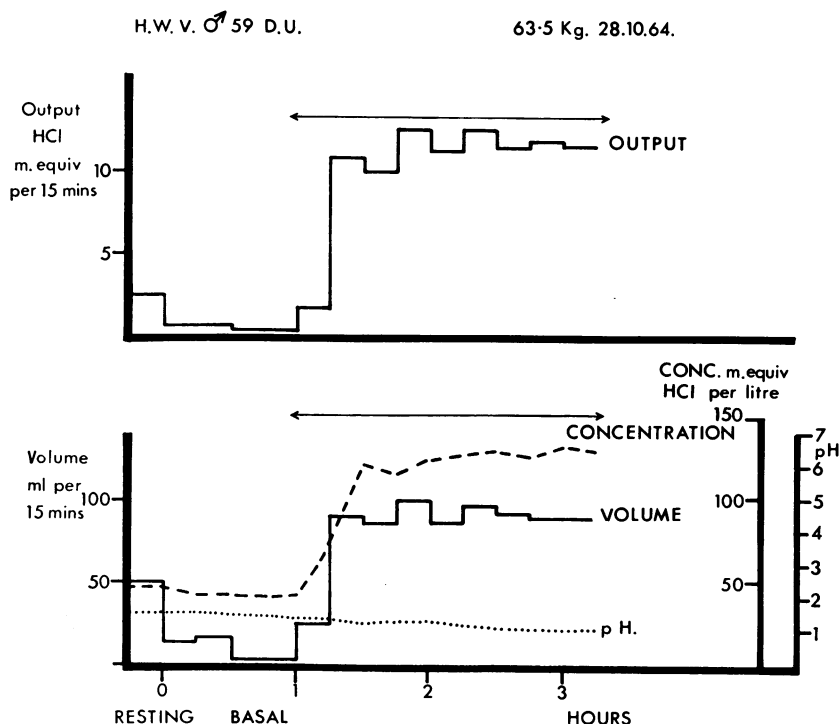


FIG. 2.—Histamine infusion test.

gates these side-effects. Further, the side-effects of histamine pass off soon after the intravenous infusion has been stopped. Outpatients are little upset by the test and are able to return to work immediately.

Nasogastric intubation of the patient is carried out in the normal way and continuous suction applied with the patient flat on his left side. A small paediatric scalp vein needle is inserted into an antecubital vein and 25 mg. of mepyramine maleate injected. A solution of histamine acid phosphate in saline is then delivered by slow-infusion pump to give a dose of 0.04 mg. per kg. body weight per hour (Lawrie and others, 1964).

The volume of gastric juice and its acidity rise during a period of about 30-45 minutes following the start of the infusion. Thereafter the 15-minute aspirations maintain a fairly constant volume and acidity and this 'plateau' response can be maintained for many hours (Fig. 2). Four successive 15-minute specimens of similar volume and acidity constitute a one-hour maximal output at this steady secretory rate.

It has been shown that the test is reproducible (Fig. 3) and in one patient recently studied the hourly outputs of HCl on four separate occasions were 42.3, 41.8, 37.9 and 39.2 mEq. In a comparison of the response of histamine infusions and augmented histamine tests in 45 patients, it was also shown that the hourly output of acid during the infusion of histamine was significantly greater than that in the augmented histamine response (Fig. 4) (Lawrie and others, 1964).

An incidental advantage of the histamine infusion test is that errors arising from incomplete aspiration of gastric contents are immediately apparent. When a steady state of secretion has been reached, fluctuation in the volume of the 15-minute collections indicates that the tube is badly placed or blocked. As the test is continued until four successive 15-minute collections are of equal value, the result is not jeopardised by such adjustments.

X-ray studies following the injection of a radio-opaque substance have confirmed that, with the patient on his left side, the maintenance of a plateau indicates satisfactory tube

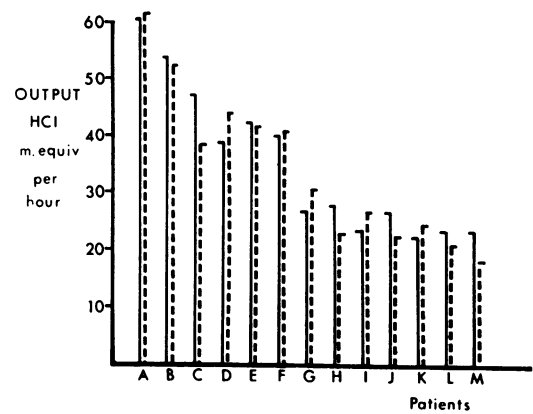


FIG. 3.—Duplicate histamine infusion tests in 13 patients. (Lawrie *et al.*, 1964). Reproduced by permission of the Editor of *The Lancet*.

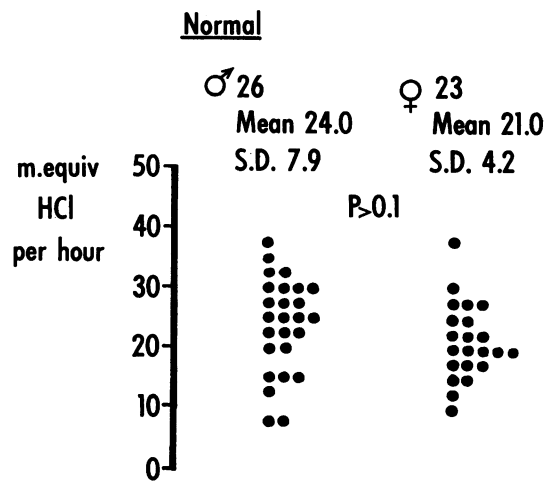


FIG. 5.—Results of histamine infusion test in 49 normal subjects.

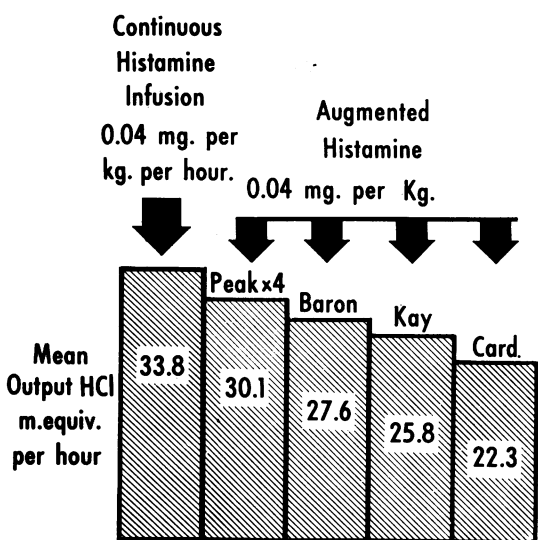


FIG. 4.—Comparison of mean output from 45 infusion tests with augmented histamine responses in patients. These have been interpreted as (1) Peak 15-minute x 4; (2) two highest successive 15-minute peaks (Baron, 1963b); (3) 15-45 minutes x 2 (Kay, 1953); and (4) 0-60 minutes. (Card and Marks, 1960)—(from Lawrie, *et al.*, 1964). Reproduced by permission of the editor of *The Lancet*.

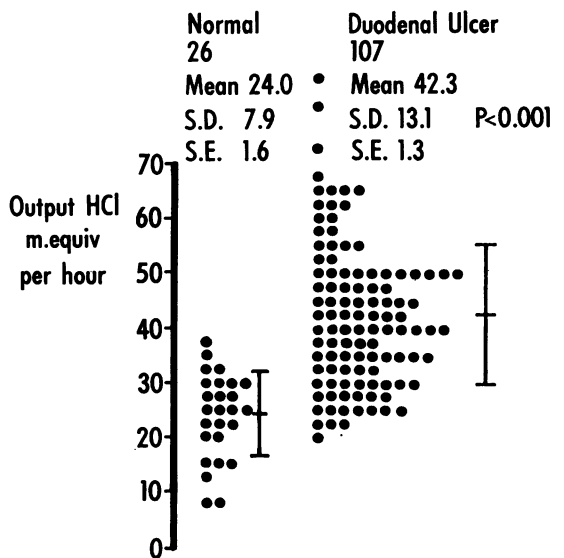


FIG. 6.—Results of histamine infusion test in patients with duodenal ulcer.

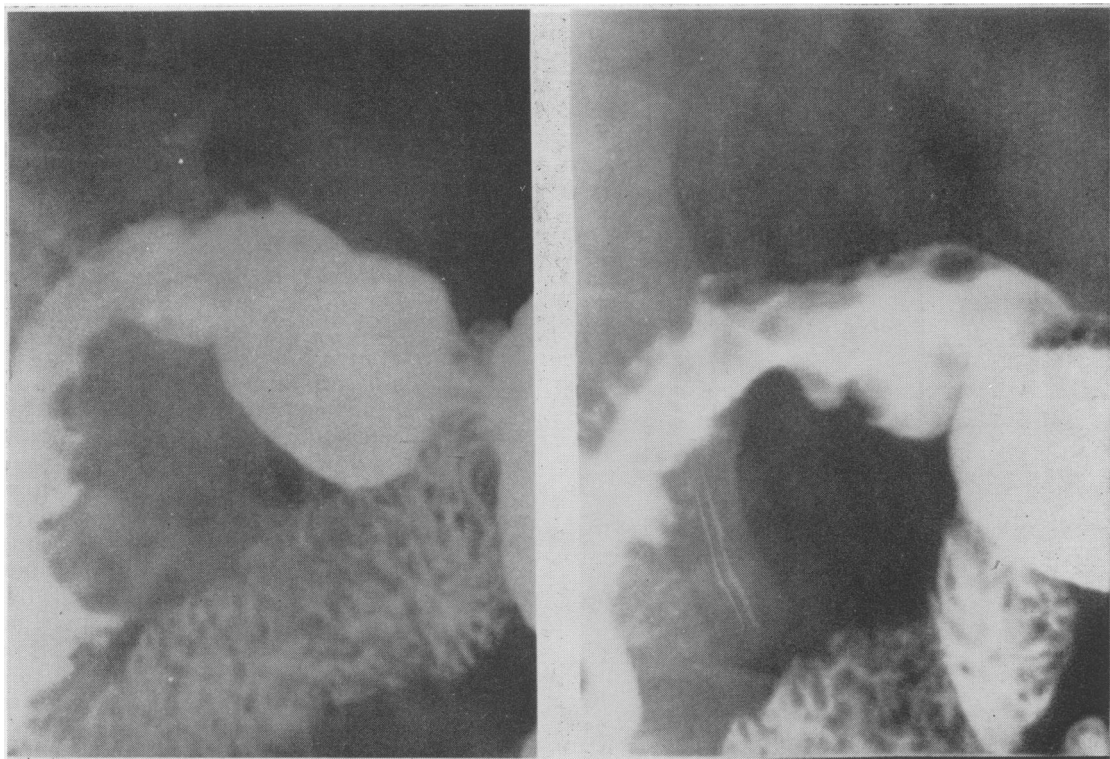


FIG. 7.—Coarse mucosal folds in duodenum shown in the supine position on the right, with the folds obliterated by filling the duodenal cap on the left.

position. As a result, screening of the patient is an unnecessary addition.

During the past two years this test has been used routinely to study gastric function in our laboratories. The results can be summarised as follows:

Normal response. The mean acid output and its standard deviation of 26 normal men and of 23 normal women was 24.0 ± 7.9 and 21.0 ± 4.2 mEq./hour respectively (Fig. 5). In contrast with other studies, in which men have had a higher output (Baron, 1963) these means are not significantly different. In our series of normal subjects, acid output falls with advancing age. Again, in contrast to the findings of others using the augmented histamine test (Baron, 1964) no correlation with body weight was observed.

Duodenal ulcer. The mean acid output in 107 male patients with proved duodenal ulcer was 42.3 ± 13.1 mEq./hour. This is significantly

different from that in normal men (Fig. 6). In the ulcer group the acid output was correlated neither with age nor body weight.

Duodenitis. During the past three years we have studied a group of 33 patients with a characteristic radiological abnormality described as 'coarse duodenal mucosal folds' (Fraser, Pitman, Lawrie, Smith, Forrest and Rhodes, 1964). The outstanding radiological feature is a cobblestone appearance of the mucosa of the first and sometimes also of the second part of the duodenum (Fig. 7). This is best seen in the supine position with the duodenum filled with air and coated with barium. Such patients have typical symptoms of peptic ulcer but no demonstrable ulcer.

The acid output of this group, estimated by the histamine infusion test, was 45.0 ± 11.8 mEq./hour, a mean not significantly different from that of patients with duodenal ulcer (Fig. 8). The findings of Rhodes, Apsimon and

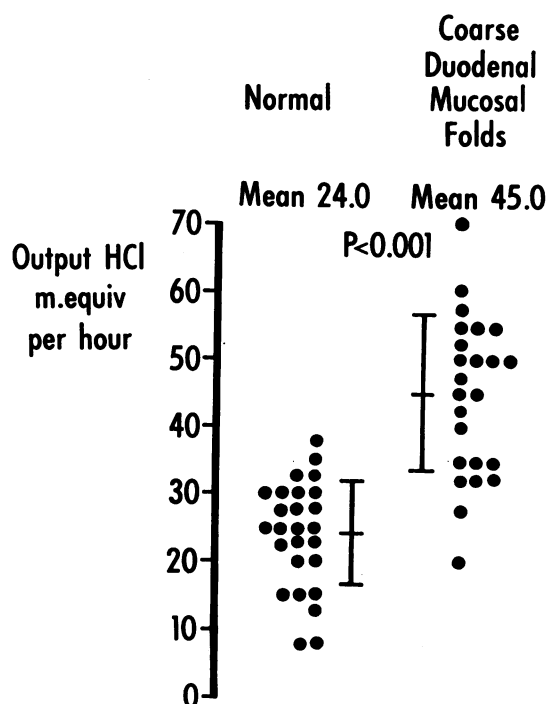


FIG. 8.—Results of histamine infusion test in 25 patients with coarse mucosal folds in the duodenum contrasted with those in 26 normal males.

Lawrie (1965) and of James (1964) also suggest that in these patients the acid environment in the duodenum is abnormally high.

Patients with this triad of (1) typical symptoms of peptic ulcer (2) a high acid output, and (3) coarse duodenal mucosal folds, cannot therefore be dismissed as functional. If their symptoms are severe and persistent, they merit definitive surgery, as for frank duodenal ulceration.

Gastric freezing. Serial histamine infusion tests were used to assess the effect of intra-gastric freezing (Wagensteen, Peter, Nicoloff Walder, Sosin and Bernstein, 1962) on parietal cell activity in 12 patients (Lawrie, Smith, Goodall, Pitman and Forrest, 1965). The results indicated that depression of secretion does not constantly occur. In two of the four patients in whom inhibition of secretion occurred, this was temporary and pre-treatment levels were regained within six months (Fig. 9).

Achlorhydria. The histamine infusion test has proved of particular value in conditions in which the output of acid is severely depressed. Hourly outputs of a few milliequivalents of HCl can be accurately assessed, allowing

discrimination between those who are truly achlorhydric and those in whom acid secretion is still present, albeit at a low level. For example, in iron-deficiency anaemia, a whole range of outputs from complete achlorhydria to normal levels have been demonstrated. Previously, such patients have been regarded as either 'normal' secretors or 'achlorhydric'.

Histalog

This analogue of histamine, a 3-beta aminoethyl pyrazole, stimulates acid gastric secretion without the usual side effects of histamine. Large doses can therefore be given without antihistamine cover.

Maximal acid outputs can be induced by a single subcutaneous injection of 200 mg. and correlate well with those of the augmented histamine test (Ward, Gillespie, Passaro and Grossman, 1960). The slightly higher outputs noted with histalog may be due to the longer duration of its effect compared with histamine.

In the United States histalog, given by a single injection, has been used to study acid gastric secretion in man (Kirsner and Ford, 1955; Grossman, Kirsner and Gillespie, 1963). These responses to single injection have the same disadvantages mentioned in relation to the augmented histamine test. Further, the relatively high cost of histalog prevents its widespread use in clinical practice.

Gastrin

With the extraction and isolation in pure form of the natural gastric secretory hormone, gastrin, by Gregory and Tracy (1961, 1964) a physiological stimulus is now available for tests of gastric function. These workers have shown that a mixture of their two polypeptides, gastrin I and II, will elicit a steady state of acid output from the stomach in dogs if given by repeated subcutaneous injection or continuous infusion. The output of acid is proportional to the dose.

Studies in human subjects are, so far, few. The secretory response of the stomach of one human subject to gastrin II has been intensively investigated by Makhlof, McManus and Card (1964a). Although gastrin II is a more potent stimulus than histamine, the maximal secretory response provided by both stimuli is remarkably similar. In 16 subjects a linear relationship was found between the maximal acid outputs induced by the two drugs ($r = 0.992$). Although recently it has been suggested that the maximal output induced by gastrin may be slightly higher than that by histamine (Makhlof, McManus and Card, 1964b), the highly significant correlation between them suggests that the

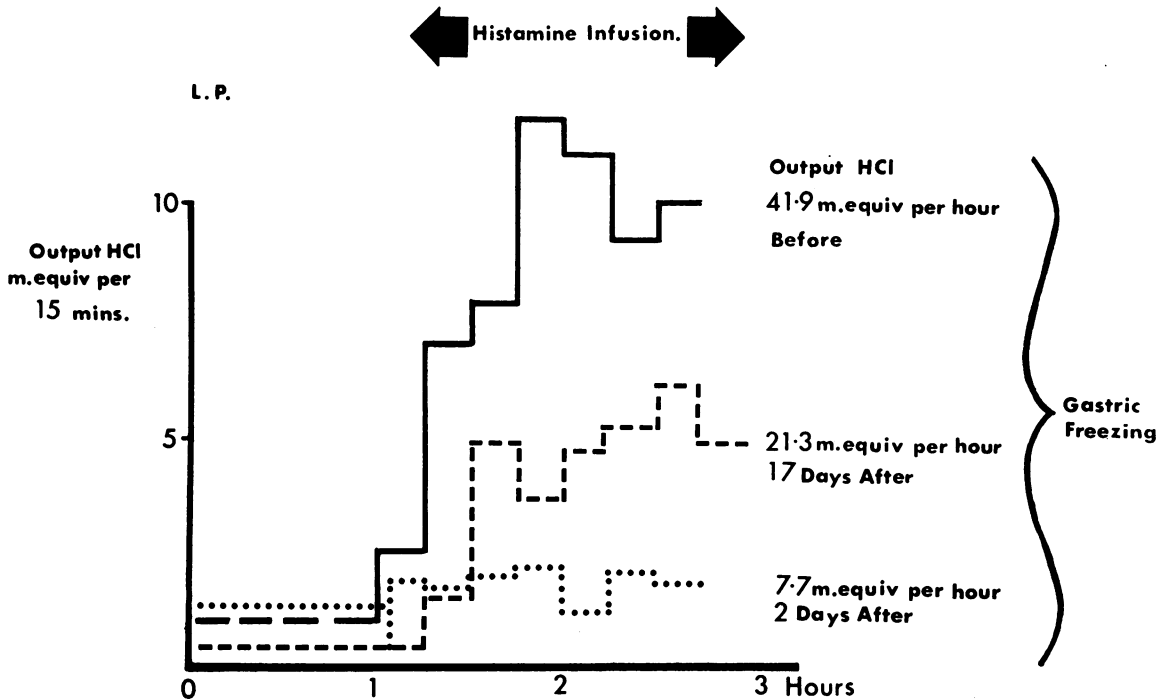


FIG. 9.—Serial histamine infusion tests in patients undergoing gastric freeze. Reproduced by permission of the Editor of the *British Journal of Surgery*.

maximal secretory response is a valid and reproducible index of gastric function, irrespective of the stimulus used.

Standardised and pure preparations of gastrin are not yet available commercially, and further definition of the place of this stimulus in the assessment of human gastric secretion must await their supply.

Conclusions

The output of acid by the human stomach can be estimated simply by the precise quantitative methods commonly used in the laboratory. By using an infusion of histamine acid phosphate as the stimulus a steady state of maximal secretion is achieved and can be measured. It is believed that this test has distinct advantages over the augmented histamine test.

In the light of the development of precise methods of studying gastric secretion the 'test meal' should be abandoned.

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